

### **Amendments to the Specification**

Please amend the identified paragraphs as follows, with additions shown underlined and deletions in strikethrough.

#### **Page 13, Paragraph beginning on Line 25:**

Fig. 8F: ~~Intra-partum AChE-R-positive granulocytes (Gran)~~ increases as a function of the increase in serum cortisol Increase in serum cortisol.

Fig. 8G: ~~Increase in serum cortisol~~ Intra-partum AChE-R-positive granulocytes (Gran) increases as a function of the increase in serum cortisol.

Asterisks indicate statistical significance.

#### **Page 51, Paragraph beginning on Line 9:**

Antibodies directed to the unique C-terminal sequences of human AChE-S [Flores-Flores (2002) *id ibid.*] and AChE-R [Sternfeld (2000) *id ibid.*] were used in conjunction with CD45 labeling to analyze the expression of the corresponding variants or fragments thereof in the sub-classified blood cell populations. Flow cytometry measurements using naive or permeabilized cells enabled distinction between cell surface and intra-cellular localization of these variants (Fig. 2C). Quantitative values were expressed as either percent positive cells (expression levels) within each population or mean fluorescence intensities of the positive fraction, which reflected content of the corresponding variant protein in each population (see below).

#### **Page 65, Paragraph beginning on Line 23:**

As mentioned before, basal levels of WBC were similar in TgR and FVB/N mice (Fig. 14A-14B

and 17B). Basal levels of ~~red blood cell counts (RBC)~~ thrombopoietin (TPO) were similar in both TgR and FVB/N mice (Fig. 17A), whereas platelet counts were significantly higher in TgR mice ( $894 \pm 87$  Vs  $1051 \pm 160 \times 10^9/\text{mL}$ ,  $p < 0.001$ ,  $n = 25$ , ~~Fig. 17B~~ Fig. 17C). Since manual differential of WBC sub-populations showed similar distributions into granulocytes, monocytes and lymphocytes in TgR and FVB/N mice (Fig. 14A-14B and 17B), the results with the platelets reflect selective thrombocytosis under chronic AChE-R overexpression.

After ip LPS injection RBC counts predictably dropped up to 72 hrs post-LPS (~~Fig. 17A~~) (Fig. 16) (in control FVB/N but not TgR mice. WBC dropped in both strains, but counts recovered considerably faster in TgR mice reaching significantly higher levels than those of FVB/N control mice by 72 hr post LPS injection ( $p < 0.02$ ,  $n = 10$ , ~~Fig. 17B and 17D~~ Figs. 16B and 16D). Platelet counts in FVB/N control mice dropped significantly, as expected, to thrombocytopenic levels between 24 and 72 hrs, while in TgR mice the platelet counts were only slightly reduced and returned to normal values within 72 hrs ( $p < 0.001$ ,  $n = 10$ , ~~Fig. 17C~~ Fig. 16C).

**Page 66. Paragraph beginning on Line 14:**

To further study the observation of elevated platelet counts in TgR mice, TPO concentrations were measured in the plasma and BM cell extracts from TgR and FVB/N mice. TPO concentrations were significantly higher in both BM and plasma of TgR mice ( $p = 0.013$ ,  $0.04$  respectively, compared to FVB/N control mice (~~Fig. 18A~~), (Figs. 17A and 17B), consistent with the notion that these mice can serve as a model of chronic inflammation [Stohlawetz (1999) *id ibid.*; Kaser A. *et al.* (2001) *Blood* **98**: 2720-2725; Zahorec R. (2001) *Bratisl. Lek. Listy*. **102**:5-14]. TgR mice BM had higher TPO levels 24 hrs post LPS injection ( $p = 0.002$ ) followed by lower TPO levels at 72 hrs ( $p = 0.02$ ,  $n = 10$ , Fig. 3A), as compared with FVB/N mice. In plasma, the high basal TPO levels were maintained 24 hrs post LPS injection ( $p = 0.01$ ,  $n = 10$ ). However, at this time point the FVB/N mice plasma TPO levels were significantly higher than TgR mice possibly due to corresponding dramatic drop in platelet numbers (~~Fig. 17C~~) (Fig. 17B). TPO levels decreased slightly but remained elevated in both mouse strains at 72 hrs (~~Fig. 18B~~) (Fig. 17B).

**Page 67, Paragraph beginning on Line 3:**

To study the possible effects of AChE-R in the inflammatory reaction, the inventors measured the levels of inflammatory cytokines in plasma and BM extracts of TgR and FVB/N mice. IL-6, but not TNF $\alpha$  levels were found to be significantly elevated in the plasma of TgR mice as was AThCh hydrolyzing activity compared with FVB/N control mice, suggesting that AChE catalytic activity might be involved in modified inflammatory control (~~Fig. 16C~~) (Fig. 15C).

**Page 67, Paragraph beginning on Line 28:**

In addition to their high baseline AChE catalytic activity (~~Fig. 16C~~) (Fig. 15C), TgR mice responded to LPS injections by a further significant increase in bone marrow AChE catalytic activity 24 hrs post LPS injection ( $p = 0.0004$ ,  $n = 10$ ), but not at other time points (Table 5B).

**Page 68, Paragraph beginning on Line 15:**

The proliferating potential of BM progenitor cells was evaluated by clonogenic assays using growth factors to support the development of the specific hematopoietic lineages. Colonies were classified as colony forming units - megakaryocyte (CFU-Mk), CFU-granulocyte/macrophage (CFU-GM) or CFU-granulocyte/erythrocyte/monocyte/megakaryocyte (CFU-GEMM) and were counted 10 to 14 days after plating. TgR mice showed significantly higher baseline numbers of CFU-Mk, -GM and -GEMM hematopoietic progenitor cells as compared to FVB/N controls ( $p \leq 0.003$ ,  $n=12$ , ~~Fig. 19A-24C~~ Figs. 18A-21C). Following LPS injection, TgR mice maintained significantly higher number of megakaryocyte progenitors ( $p < 0.0002$ ,  $n = 12$ , ~~Fig. 19A~~ Fig. 18A). In FVB/N mice, the number of CFU-GM, was significantly elevated at 24 hr post-LPS, a response previously described [Peterson (1992) *id ibid.*; Yokochi (1985) *id ibid.*] ( $p = 0.01$ ,  $n = 12$ ,

~~Fig. 49B~~ Fig. 18B) but decreased noticeably by 48 hr, while TgR CFU-GM numbers decreased 48 hr post LPS injection but remained significantly higher than FVB/N ( $p = 0.03$ ,  $n = 12$ , ~~Fig. 49B~~ Fig. 18B). The increase in CFU-GM in TgR mice was less dramatic than in FVB/N control mice perhaps caused by fatigue of myeloid progenitor cells due to chronic exposure to AChE-R. TgR and FVB/N mice showed similar post-LPS numbers of multipotential CFU-GEMM (NS,  $n = 12$ , ~~Fig. 49C~~ Fig. 18C).

**Page 69, Paragraph beginning on Line 3:**

AChE-R was reported to interact with the scaffold protein RACK1 and with its target, protein kinase C  $\beta$ II (PKC  $\beta$ II) [Birikh K.R. *et al.* (2003) *Proc. Natl. Acad. Sci. U S A* **100**:283-288; WO 00/73427] or PKC  $\epsilon$  [Perry C. *et al.* (2004) *Neoplasia* **6**(3):279-86]. PKC $\epsilon$  has been implicated in the programming of megakaryocytic lineage commitment and potentiates the transcription factor GATA-1 [Racke F.K. *et al.* (2001) *J. Biol. Chem.* **276**:522-528]. To study a potential AChE-R/PKC $\epsilon$ /RACK1 interaction in megakaryocytes, AChE-R, PKC $\epsilon$  and RACK1 were detected in BM smears of TgR and FVB/N mice (Fig. ~~20A-20F~~ 19A-19F).

Megakaryocytes were detected in BM smears by the May-Grünwald staining (Fig. ~~20A~~ 19A). TgR megakaryocytes predictably expressed higher AChE-R labeling than megakaryocytes from FVB/N mice ( $212.3 \pm 15.0$  Vs  $130.9 \pm 18.3$  luminescence units,  $p < 10^{-11}$ ,  $n = 50$ , ~~Fig. 20B, 20F~~ Figs. 19B, 19F and Table 3). Intriguingly, RACK1 labeling intensity was discernable, although insignificantly elevated in TgR megakaryocytes, as compared to FVB/N mice ( $162.3 \pm 49.2$  Vs  $153.4 \pm 21.0$ , NS,  $n = 50$ , ~~Fig. 20C, 20F~~ Figs. 19C, 19F and Table 6). No differences in the number of PKC  $\epsilon$ -labeled megakaryocytes were detected in TgR mice (data not shown), nevertheless, the intensity of PKC  $\epsilon$  labeling was significantly higher as compared to FVB/N mice ( $187.7 \pm 22.2$  Vs  $160.9 \pm 19.7$  luminescence units,  $p < 10^{-5}$ ,  $n = 50$ , ~~Fig. 20D, 20F~~ Figs. 19D, 19F and Table 6). Thus, AChE-R interaction signaling with RACK1 and with PKC $\epsilon$  emerged as a putative mechanism for increased intracellular signaling in TgR megakaryocytes.

**Page 70, Paragraph beginning on Line 13:**

TgR mice elevated platelet counts and increased megakaryocyte growth potential was suggestive to determine whether AChE-R, or its cleavable peptide ARP, can improve engraftment of transplanted BM cells and recovery from thrombocytopenia in a NOD/SCID mouse transplantation model. Human CB CD34<sup>+</sup> cells were primed for 2 hrs prior to injection with ARP<sub>26</sub>, a synthetic peptide comprised of 26 amino acids of the C-terminal sequence of AChE-R or ASP<sub>40</sub> a 43 amino acid sequence of the C terminus of AChE-S. The ARP concentration chosen (2nM) was previously determined to be maximal for stimulating hematopoietic stem cell proliferation [Deutsch (2002) *id ibid.*] Human CB CD34<sup>+</sup> cells (1x10<sup>5</sup>) were injected into mice 24 hours post irradiation. Cells were either primed and supplemented with ARP<sub>26</sub> or primed and supplemented with ASP<sub>40</sub> or untreated (control). Mice were sacrificed 6 weeks post-transplantation and single cell suspensions from BM extracted from the femur bones assessed for the presence of human hematopoietic cells. Monoclonal antibodies against human CD45, CD34 and CD41 were used to assess engraftment efficacy of the transplanted human cells. Fractions of human CD34<sup>+</sup> cells in the BM of NOD/SCID mice post transplant were similar in all groups (~~Fig. 21A~~) (Fig. 20A). However, significantly more human CD45<sup>+</sup> cells were found in the BM of NOD/SCID mice injected with ARP<sub>26</sub> together with ARP<sub>26</sub>-primed CD34<sup>+</sup> cells (p = 0.02, n = 12, 16 and 8 mice, respectively, ~~Fig. 21A~~ Fig. 20A). Fractions of human megakaryocytes (CD41<sup>+</sup>) were higher in the BM of NOD/SCID mice that received ARP<sub>26</sub>-primed cells as compared with ASP<sub>40</sub> -primed or non-primed human cells (p = 0.03, n = 12, 16 and 8 mice, respectively, ~~Fig. 21A~~ Fig. 20A). These results demonstrate a significantly better engraftment of transplanted primed human CD34<sup>+</sup> cells when injected with ARP<sub>26</sub> as compared with non-ARP<sub>26</sub> treated cells.

Quantitative PCR with human specific probes was used to assess the relative presence of human DNA in the BM of NOD/SCID mice. Significant differences could be observed between mice transplanted with ARP<sub>26</sub>-primed CD34<sup>+</sup> cells as compared to cells primed with ASP<sub>40</sub> or non-primed cells (p = 0.015, ~~Fig. 21B~~ Fig. 20B).

**Page 71, Paragraph beginning on Line 20:**

In an attempt to improve platelet recovery in NOD/SCID mice the number of committed megakaryocyte progenitor cells in the stem cell graft were expanded ex-vivo. CD34<sup>+</sup> cells were incubated for 10 days in medium supplemented with 10% plasma and one of the following combinations: ARP<sub>26</sub>, (2nM) ASP<sub>43</sub> (2nM), TPO (10 ng/ml) and SCF (50 ng) (growth factors optimal for megakaryocyte commitment) or no growth factor supplement (control). CD34<sup>+</sup> cells are known to differentiate in culture producing many committed progenitors, but have reduced capacity for long-term engraftment in NOD/SCID mice. Therefore, freshly isolated CD34<sup>+</sup> cells are needed to enable long-term engraftment [Guenechea G. *et al.* (1999) *Blood* **93**:1097-1105; Li K. *et al.* (1999) *Br. J. Haematol.* **104**:178-185]. For this reason a mixture of cultured CD34<sup>+</sup> cells (between 1-2x10<sup>5</sup>) was injected together with 100,000 fresh CB CD34<sup>+</sup> cells. The cultured CD34<sup>+</sup> cells, being more mature were expected to facilitate the capacity for early platelet production. Early engraftment (2-3 weeks post-transplant) and late engraftment (4 and 6 weeks post-transplant) were analyzed. Incubating CD34<sup>+</sup> cells with ARP<sub>26</sub>, ASP<sub>40</sub> or TPO and SCF did not augment engraftment of human CD45<sup>+</sup>, CD34<sup>+</sup> or CD41<sup>+</sup> cells (~~Fig. 22A~~) (~~Fig. 21A~~) however, it enabled to test whether the injected differentiated cells affected platelet production. Full blood cell counts were performed on the transplanted NOD/SCID mice and the presence of human platelets monitored. Although significant differences were not found, probably due to the small sample number of mice (n=6), NOD/SCID mice that received cells expanded with ARP<sub>26</sub> yielded higher human platelet numbers, both early (between 2 and 3 weeks post-transplant) (mean =1.26 control Vs 3.29 ARP<sub>26</sub> expanded Vs 0.94 ASP<sub>40</sub> expanded Vs 1.61 x 10<sup>6</sup>/ml TPO/SCF expanded group, ~~Fig. 22B~~ Fig. 21B) and at the late transplanted stage (mean = 5.85 control Vs 17.70 ARP<sub>26</sub> expanded Vs 6.39 ASP<sub>40</sub> expanded Vs 3.44 x 10<sup>6</sup>/ml TPO/SCF expanded group, ~~Fig. 22B~~ Fig. 21B). These observations were compatible with the hypothesis that the injected differentiated megakaryocytes facilitated platelet production in the engrafted mice and that the enhanced AChE-R production by these cells support a shift towards megakaryocytopoiesis, which culminated in higher platelet counts at the later test time.